the outstanding Office action and authorization to charge the appropriate fee are enclosed herewith.

In response to the Raw Sequence Error Report dated September 18, 1997 that was included with the present Office Action, Applicants have submitted a copy of the Report, an amended sequence listing including the oligonucleotide primer sequences of SEQ ID NOS: 1-3, an amendment to the description corresponding to the amended sequence listing, and a computer readable copy of the amended sequence listing in accord with requirements of 37 CFR §§ 1.821-1.825.

Also enclosed is a "Statement in Support of Filing and Submissions In Accordance with 37 CFR 1.821-1.825", in which I declare that the content of the paper and the computer-readable copies of the Sequence Listing submitted in accordance with 37 CFR 1.821 (c) and (e), respectively, are the same and that the submission, filed in accordance with 37 CFR 1.821 (g) does not introduce new matter.

The claims have been amended to more particularly define the present invention. Support for these amendments is found throughout the specification. No new matter has been added by virtue of the amendments made to the claims.

Claims 1-6, 25-52 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that applicant regards as the invention. The rejection is respectfully traversed with respect to claim 1, 2 to 6 and 39 to 52.

In formulating this rejection, the Action states at page 2 that:

> Claims 1-6 and 25-52 are confusing with respect to the names provided, or rather, not provided to the various primers which are used in the claimed method. With no clear indication as to which primer is being used at any given time, and with the primers being referred to as "said primer," it is rather confounding as to just how many primers are in the reaction.

Applicant has amended claim 25 to more precisely define the oligonucleotide primers recited therein. Dependent claims 26, 27, and 30 have been amended to maintain proper antecedency with claim 25. It is believed that the amended claim now unambigously recites the primers used in the method. Accordingly, reconsideration and withdrawal of the rejection as it applies to the amended claims is requested.

Applicant respectfully disagrees with the present rejection as it applies to claim 1 to 6 and 39 to 57. Claims 1-4, 7, 39-41, 44, 53, 54 and 57 recite an oligonucleotide primer. It is believed that recitation of one oligonucleotide primer in each of the claims is unambigous and would be clearly understood by an artisan working in this particular field.

It is respectfully submitted that the remaining claims are definite under 35 USC §112. The terms "oligonucleotide primer" and "an extended oligonucleotide primer" are distinct and have clear meaning to the skilled artisan.

In the above-captioned remarks, the position seems to have been asserted that the claims should be changed to point out how many primers are in a reaction. Applicant respectfully disagrees with that assertion. It is not necessary to recite numbers of primers in order to satisfy § 112, second paragraph. The artisan working in this particular field would know that the claimed methods can be practiced with one or more primers capable of forming extension products. See e.g., Figures 2 and 3 and supporting disclosure in the specification. Accordingly, reconsideration and withdrawal of the rejection is requested.

Claims 4, 11, 27, 41 and 53 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The rejection is respectfully traversed.

In formulating this rejection, the Action states at page 2 that:

> Claims 4, 11, 27, 41, and 53 are indefinite with respect to just what constitutes "substantially." claims 5 and 6 which depend from claim 4; claims 12 and 13 which depend from claim 11; claims 28 and 29 which depend from claim 27; claims 42 and 43 which depend from claim 41; and claims 54-57 which depend from claim 53 fail to overcome this issue and are similarly indefinite.

The word "substantially" appears in the cited claims as "substantially identical". It is submitted that the phrase "substantially identical" is not vague or indefinite in view of the disclosure provided by Applicant's specification. For example, on page 26, lines 13-20, Applicant has specifically defined the phrase to mean two sequences which have at least 90% of the same or analogous base sequences where thymine (T) and uracil (U) are considered the same. That definition is believed to fully satisfy the requirements of §112, second paragraph. Withdrawal of the rejection is respectfully requested.

Claims 1-57 stand rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent Nos. 4,683,202 (hereinafter "Mullis"); 5,391,480 (hereinafter "Davis") 5,618,664 (hereinafter "Kiessling"); 5,627,054 (hereinafter "Gillipsie"). The rejection is respectfully traversed.

In formulating this rejection, the Action states at pages 3 to 5 that:

> Mullis teaches at length conditions for performing polymerase chain reaction (PCR), including the selection and manufacture of primers; the types of sources from which a nucleic acid sample can be obtained; temperature cycling; repeated cycles of primer extension, annealing, dissociation, etc.; the performance of PCR with nested primers; see column 25, Example 10. Mullis does not disclose the use of a primer which has a 3' terminal portion which does not anneal to a target/control sequence and which is selectively cleaved with a 3' to 5' exonuclease.
>
> Kiessling, column 13, penultimate paragraph, discloses performing PCR where one incorporates into the reaction a positive control nucleic acid sequence. Kiessling also discloses kits that can be formatted; see column 8, first full paragraph. Kiessling does not disclose the use of primers which have an intentionally non-annealing 3' terminal portion and which is selectively cleaved with a 3' to 5' exonuclease.
>
> Davis et al., disclose the use of a primer which has an intentionally non-annealing 3' terminal portion which is cleaved through the action of a 3' to 5' exonuclease; see Fig. 2.

As seen in column 2, first full paragraph, and at column 5, lines 44-45, the use of a 3' to 5' exonuclease is preferred to remove the non-annealing terminal nucleotide. Column 6 discloses a variety of detectable labels that may be incorporated into the nucleotide. Davis et al., do not define the non-annealing terminal portion of the primer a being that which is from 1 to 10 nucleotides in length.

Gillespie disclose the use of two primers where one of said primers has an intentionally non-annealing 3' portion. Column 4, first full paragraph states that the primer is 20-50 nucleotides in length and with a non-complementary portion that is preferably 5-10 nucleotides in length.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to have combined the primer disclose by Gillepsie with the method of Davis et al., Kiessling and Mullis so to achieve a positive control for a PCR reaction where one would be able to determine if the control primer was functioning in the manner intended, and by extension, determine if the PCR reaction as a whole was proceeding correctly. In view of the guidance provided in the art, and the explicit recitation of using primers which have an intentional non-annealing overhang in such PCR reactions, the ordinary artisan would have been motivated to have combined such teachings and to have done so with a reasonable expectation of success. The aspect of combing the requisite reagents in a kit as disclosed by Kiessling would have been an obvious commercial expediency, requiring little of any additional research and development.

Applicant respectfully disagrees with the rejection. It is believed that the rejection represents hindsight reconstruction of the Applicant's invention and that there would be no motivation to combine the references in the way the Examiner has done.

Gillepsie teaches competitive primers that block primer hybridization (ie. annealing) and initiate asymmetric amplification of a target sequence. Preferred competitive primers according to Gillepsie are incapable of extension themselves by virtue of a 3' overhang sequence that is preferably between 5-10 nucleotides in length. See e.g., Gillepsie at col. 3, lines 13-23; and col. 4, lines 11-19.

In contrast to the competitive primers of Gillepsie, the primers of the present invention are essentially non-competitive, ie., the primers do not significantly impede hybridization of other primers in the claimed methods. See e.g., Figures 1, 1A, 2 and 3 and supporting disclosure on pages 30-35.

Since Gillepsie's primers are disclosed as being competitive, it is believed that the method formulated by the Examiner's combination of the cited references (hereinafter "prior method") would be a competitive PCR amplification method. The prior method is thus completely different from the essentially non-competitive method of the claims. See e.g., Figures 1, 1A, 2 and 3. In formulating the rejection, the Examiner has neglected the competitive characteristics of Gillepsie's primers while highlighting the reported inability of those primers to anneal at the 3' end. This "cherry picking" of the Gillepsie is improper and represents hindsight reconstruction of Applicant's invention.

Additionally, Gillepsie's competitive primers are reported to initiate asymetric amplification of a desired template, whereas use of primers in accord with the claimed methods can lead to formation of double-stranded amplification products. See e.g., Gillepsie at Figure. 1 and col. 3, lines 13-23; and the present application at Figures 1, 1A and 2.

In addition to these significant differences, the prior method is not the Applicant's invention.

For example, Kiessling discloses use of a single plasmid DNA as a control template. Thus, the prior method includes one control template for PCR amplification. In contrast, the present methods are capable of amplifying multiple templates in the same reaction. Neither Kiessling nor any other of the references as cited by the Examiner taken individually or in combination teach, suggest or provide any motivation to amplify multiple templates in the same reaction. Even if the artisan were motivated to amplify more than one template in the same reaction in the prior method, the cited references do disclose or suggest how to control PCR amplification in a way that achieves regulated amplification of one template relative to another template. As disclosed in the present application, that goal is achieved in one embodiment by using a primer for amplification of polynucleotide templates where the primer has a 3'-mismatch with respect to its binding site on one of the templates. See e.g,. pages 6-7, bridging paragraph.

As a further illustration of the non-obviousness of the present invention, Figure 1 shows one embodiment in which amplification of a test polynucleotide (TPN) is controlled relative to a control polynucleotide (CPN). In this particular example, amplification of the TPN proceeds by extension of two primers (PP1, PP2) to produce extended templates (EPP1, EPP2). Amplification of the CPN is controllably suppressed relative to the TPN due to a 3' overhang sequence on PP1 that regulates both extension of PP1 and amplification of CPN. That ability to controllably suppress amplification of the CPN is one important feature that distinguishes the present invention from the prior method. In particular, the supression has been found by Applicant to prevent the CPN from consuming so much of the amplification reagents that amplification of the TPN is inhibited. The 3' overhang sequence is removed by an exonuclease (NP-Exo) to allow amplification of the CPN by PP1. See also, pg. 30, line 22 to pg. 31, line 20; pg. 12 line 18 to pg. 13 line 11.

Further, Applicant has found that the amount and timing of the controlled amplification method illustrated in Figure 1 (and elsewhere in the application) is a direct reflection of the concentration of the TPN. The amplification method thus provides for qualitative or a quantitative determination of the control sequence CPN and in addition, indicates that amplification reagents and conditions in the reaction are functional. See e.g., pg. 7, lines 5-8; pg. 12, lines 1-17; and pg .13 lines 22-24.

Nowhere in the cited references either taken individually or in combination, can Applicant find any teaching or suggestion of Applicant's method of providing for controlled amplification of multiple polynucleotide templates.

In addition , Applicant can find no teaching or suggestion in the cited references of Applicant's method of providing qualitative or quantitative determination of multiple templates and of providing an indication of functional amplification conditions.

For reasons already mentioned above, the kit of claims 53-57 is also unobvious in view of the Examiner's combination of references.

In view of the above discussion, it is respectfully submitted that the outstanding rejections have been overcome and should be withdrawn. Early reconsideration and notice of allowance are earnestly solicited.

Should the Examiner wish to discuss any of the amendments and/or remarks made herein, the undersigned attorney would appreciate the opportunity to do so.

Respectfully submitted,

Date: 1/30/98

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